11.2 μl; DMSO, 8.6 μl; 6 mM dNTP, 3.0 μl; common forward primer (50 pmole), 2.0 μl; normal reverse primer (50 pmole), 2.0 μl; genomic DNA, 1–4 μl (0.2 μgram). A second reaction mixture for the detection of the mutant allele is prepared the same way, except that the normal reverse primer is replaced by the mutant reverse primer (50 pmole), 2.0 μl. Denature initially for 5–7 min at 99°C; hot-start at 85°C, at which time 2.5 U (=0.5 μl) Taq enzyme (in 5 μl of reaction mixture) are added to each tube. The amplification profile is as follows: denaturation at 95°C (1 min), annealing at 68°C (1 min), and elongation at 72°C (2.5 min) for 25 cycles. It is not advisable to increase the amount of Taq enzyme above 2.5 U. Detection of amplification products is similar to our description in the original paper [1].

As stated, this new set of primers has given us consistently satisfactory data; differentiation between types I and II of the 3.7-kb deletion after *Apa*I digestion of the PCR product can also be obtained with this modified procedure. We have not encountered any such problems with the detection of the 4.2-kb deletion.

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Prenatal Diagnosis Based on Simultaneous DNA Analysis for α- and β-Globin Genes

To the Editor: β-thalassemia is the prototype of a single-gene disorder. The majority of β-thalassemia patients show a phenotype which can be directly explained by genotype (severity of mutations). However, a number of clinically significant interactions between α-globin gene abnormalities and β-thalassemia have been described, which can lead to major modifications of phenotype. We report here on the performance of prenatal diagnosis for symptomatic β-thalassemia intermedia, which required analysis-in-parallel of both β-globin and α-globin genes.

Over a decade ago, Wainscoat et al. [1] noted that the phenotype of β-thalassemia patients can be significantly ameliorated by the coexistence of α-thalassemia. On the other hand, symptomatic β-thalassemia has been shown to result from the interaction of heterozygosity for β-thalassemia with excess α-globin genes [2,3]. The carriership of α-globin gene triplication does not affect hematologic parameters. Therefore, individuals who carry this rearrangement will not be identified as at risk for having offspring affected with thalassemia.

A Christian Arab child was referred for molecular diagnosis because of unexplained severe thalassemia intermedia, requiring regular transfusions (once every 6 weeks since the second year of life) for maintenance of normal growth. The father has β-thalassemia trait (Hb 11.5 g/dl, MCV 63.9 fl, MCH 18.9 pg; HbA2, 4.9%), while the mother is hematologically nomal (Hb 13.2 g/dl, MCV 89.8 fl, MCH 29.1 pg; HbA2, 2.9%). The child was found to be heterozygous for β^0-thalassemia, carrying the mutation IVS1 nt1 G-A, which he inherited from his father. Sequence analysis demonstrated that the other β-globin allele was normal. Further investigation showed that his severe phenotype was due to homozygosity for α-globin gene triplication ($\alpha\alpha\alpha^{anti\text{-}3.7}/\alpha\alpha\alpha^{anti\text{-}3.7}$ instead of the normal genotype αα/αα) [2]. Both parents are heterozygous for the α-globin gene triplication. Sequence analysis verified that the mother does not carry any β-globin mutation, and that the father carries the β^0-thalassemia mutation.

During a subsequent pregnancy, prenatal testing was requested by the parents. Fetal DNA was obtained from chorionic villi. Analysis for the IVS1-1 mutation indicated that the fetus was heterozygous (Fig. 1A). Based on this result alone, the fetus would have been diagnosed as thalassemia minor. However genomic α-globin gene analysis of fetal DNA demonstrated homozygosity for α-globin gene triplication ($\alpha\alpha\alpha^{anti\ 3.7}/\alpha\alpha\alpha^{anti\ 3.7}$, Fig. 1B). As the genotype was identical to that of the affected child, the parents elected to terminate the pregnancy.

This case exemplifies a prenatal test which required the analysis of two genes to diagnose what is considered to be a paradigm for a single gene disorder. As the complex factors affecting the phenotype of genetic diseases are clarified, multigene analysis will become more common in the future.

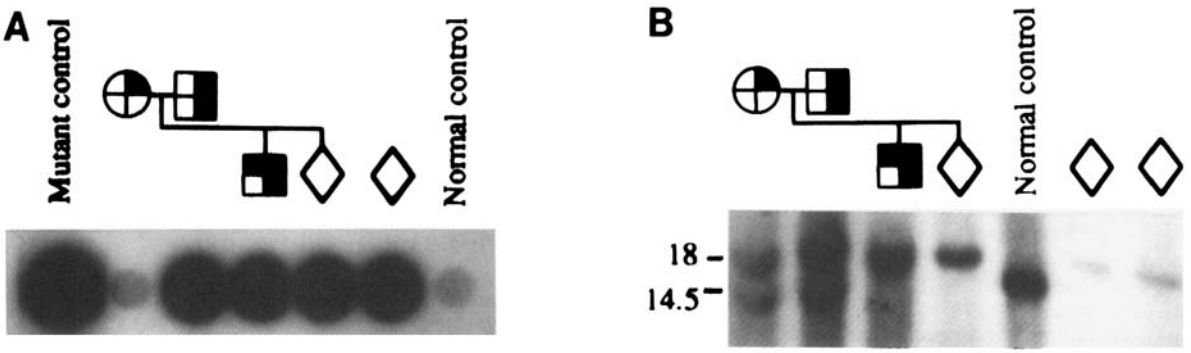

Fig. 1. A: Analysis of the IVS1-1 mutation. DNA isolated from chorionic villi was amplified by PCR using β-globin gene primers [4]. PCR products of the fetus, the propositus, and the parents were dot-blotted together with control samples onto a nylon membrane (Zeta-Probe, Bio-Rad, Hercules, CA), and hybridized to a ^{32}P-labeled IVS1-1 mutant oligonucleotide probe. The two fetal DNA samples shown were amplified separately. $\alpha\alpha\alpha^{anti\text{-}3.7}$ is denoted by stippled symbols, and solid symbols designate IVS1-1 mutation. B: Southern blot analysis of α-globin locus. Fetal DNA obtained from cultured fibroblasts was digested with *Bam*HI, in parallel to DNA of family members and a control. Hybridization was performed with a ^{32}P-labeled α-globin-specific probe [5]. Normal expected fragment size is 14.5 kb (αα). An 18-kb fragment represents a chromosome carrying the triplication ($\alpha\alpha\alpha^{anti\text{-}3.7}$). Three fetal DNA samples were digested and analyzed in parallel. Genotype symbols are as described for A.

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Gancyclovir-Induced Megakaryocyte Loss in Chronic Myelogenous Leukemia Post Bone-Marrow Transplant

To the Editor: Reversible neutropenia and, less frequently, thrombocytopenia develop in patients receiving gancyclovir, an antiviral agent effective in cytomegalovirus (CMV) infections [1–5]. The mechanism(s) of hematologic toxicity has not been well-characterized; however, it has been suggested that gancyclovir is toxic to megakaryocytes. We describe a patient with chronic myelogenous leukemia (CML) following allogeneic bone-marrow transplant (BMT) who received gancylcovir; the morphologic findings seen in this case support this hypothesis.

The patient, a 37-year-old white male, presented with weakness and malaise for several months and recurrent otitis media. A CBC showed a WBC of 170,000 with 1% myeloblasts. Four months after beginning therapy (hydroxyurea and interferon), the patient received high-dose chemotherapy followed by total-body irradiation and BMT. Both patient and donor were CMV-positive. Gancyclovir (350 mg IV, 3 times per week) was started 1 week after discharge. One month after discharge the patient was briefly hospitalized for nausea, vomiting, and abdominal pain; rectal biopsies demonstrated graft vs. host disease. His platelet count was 81,000. He was discharged on cyclosporin, zantac, coumadin, gancyclovir, fluconazole, septra, IV immunoglobulin, and prednisone. Six weeks after discharge, the patient had a platelet count of 13,000; WBC was 6.6 and his hemoglobin/hematocrit were 8.8 g/dl/26.1%. He received platelet transfusions; gancyclovir was discontinued.

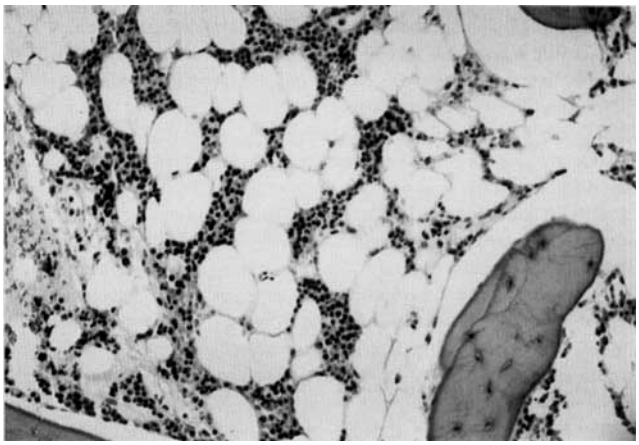

Fig. 1. Bone-marrow biopsy while patient was on gancyclovir. There was marked decrease in megakaryocytes during gancyclovir regimen (H&E, ×200).

Following discontinuation of gancyclovir, his platelet count gradually improved to over 100,000 by 6 months after transplant.

Prior to therapy, a bone-marrow biopsy showed 100% cellular marrow with 7% myeloblasts with eosinophilia. Cytogenetic studies demonstrated a Philadelphia chromosome, and Southern blot analysis identified a rearrangement of the *bcr* gene. One week after discharge, while receiving GM-CSF, a bone-marrow biopsy showed 70% cellular marrow with recovery of all bone-marrow elements. Neither Southern blot nor RT-PCR analysis of the bone marrow detected a *bcr/abl* rearrangement. Three weeks after discharge while the patient was on prophylactic gancyclovir, a bone-marrow biopsy demonstrated 20% cellular marrow with an M:E ratio of 3:1; 2% of cells were myeloblasts. There was a marked decrease in the number of megakaryocytes (Fig. 1); the megakaryocytes identified had a normal morphology.

Subsequent to discontinuation of gancyclovir, marrow cellularity increased to 25% at 6 months and 40% at 1 year posttransplant. The M:E ratio was 3:1 and 2:1 at 6 months and 1 year, respectively. The number of megakaryocytes returned to normal.

In this patient, serial bone-marrow biopsies were examined following an allogeneic bone-marrow transplant for CML. No evidence of recurrent disease was identified in any of the posttransplant biopsies. After reconstitution of all bone-marrow lineages, the patient was begun on gancyclovir prophylaxis. Subsequently he developed a hypocellular marrow with pronounced megakaryocyte hypoplasia. After discontinuation of gancyclovir, the patient's thrombocytopenia markedly improved; this was accompanied by a corresponding increase in megakaryocytes within the biopsies. These morphologic findings, while not definitive, are most consistent with a lineage-specific direct toxic affect on the bone marrow.

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